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I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES, hereby certify that the annexed is a true copy of the Provisional specification in connection with Application No. PP 2915 for a patent by ALLRAD 3 PTY LTD and PTI INVESTMENTS PTY LTD filed on 14 April 1998.

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ORIGINAL

PROVISIONAL SPECIFICATION FOR AN INVENTION ENTITLED

Invention Title: FOOD SUPPLEMENT

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The invention is described in the following statement :

This invention relates to a food supplement, derived from fruit or vegetable fibres, which has beneficial effects for bowel health.

BACKGROUND OF THE INVENTION

In the processing of fruit and vegetable for consumption, a considerable amount of the fruit or vegetable remains unused because it is either unpalatable or inconvenient to use. This represents a somewhat inefficient use of resources and leads to a waste disposal problem and a loss of potentially valuable resources.

It is also desirable to have a fibre additive for foods that is a substitute, or a partial substitute for ingredients of commonly used foods substances such as flour in bread. Also desired is that these substitutes do not add to the calorific content of the foods, and in many instances that these substitutes do either not contribute flavours at all or at least do not contribute off flavours. A number of examples of fibre food additives are made from waste from fruit or vegetable processing, and one such example is the use of treated citrus albedo for inclusion of a flour substitute in various cereal products such as bread in US patent No 4526794 by Altomare et al.

DISCLOSURE OF THE INVENTION

It is a finding of this invention that the use of a mixture of fibre extracts from two or more types of fruit or vegetables can have a beneficial effect on the large bowel.

Fibre extracts from peeled apple slices and from the albedo of oranges were extracted by a counter current method and added as a supplement to a standard feed for pigs. A startling increase in indicators of gut health was found when mixtures of the two fibre extracts were used when compared to the use of each fibre extract separately.

The effect is manifest in an increased production of short chain fatty acids in the large bowel, of which butyrate is the fatty acid that is increased to the greatest degree. The experiments conducted to date are suggestive that the physiology of the large intestine is also somewhat modified in so far as the gut wall of the large intestine is thickened albeit by a statistically not significant amount, indicating that there may be stimulation of growth.

The term fibre in the context of this invention is intended to convey the meaning of material that is relatively indigestible in the small intestine such that it passes into the large bowel of a human or other omnivorous animal species.

It is thus proposed that in a broad form that the present invention could be said to reside in a food supplement, said food supplement derived from fibre extracts from two or more types of fruit or vegetables, the fibre extracts having had a majority of soluble solids removed therefrom.

At present the reason why such combinations of fibre extracts exert their effect is unknown, it is however thought that removal of a majority of the soluble solids is essential for this to have effect. One hypothesis is that insoluble fibre components presented in this way have a more beneficial action in promoting colonisation of beneficial bacteria in the large intestine, thereby acting as a prebiotic.

The removal of soluble solids also has the side benefit of maximising the potential value obtained from the precursor product in so far as it may be possible to sell some or all of the soluble solids. Additionally the insoluble solids that remain are more convenient for food use because they may be dried and hence put into a wider range of foods than would be possible with soluble materials. Insoluble solids from which soluble solids have been removed also have a tendency to be more stable microbiologically and not to produce off flavours, there is also the possibility that any anti microbial substances (that might otherwise adversely affect beneficial large bowel microflora) present in parts of the fruit are also removed.

The two or more types of fruit or vegetable may be selected from the group consisting of grape, citrus, apple, tomato, carrot, mango, papaya, banana, pineapple, kiwi fruit, spinach and melon.

Preferably the two or more types of fruit or vegetables are selected from the group consisting of, grape, orange, apple, tomato and melon

In an alternative form a first of the two or more fruit and vegetables is a citrus fruit and a second fruit is selected from the group consisting of grape, apple, tomato, carrot, mango, papaya, banana, pineapple, kiwi fruit, spinach, a melon and more preferably the second fruit is selected from the group consisting of grape, apple, tomato and melon. In one convenient form the citrus fruit is an orange

In another alternative form a first of the two or more fruit and vegetables is an apple, and a second fruit is selected from the group consisting of grape, citrus, tomato, carrot, mango, papaya, banana, pineapple, kiwi fruit, spinach, a melon, and more preferably the second fruit is selected from the group consisting of grape, citrus, tomato, and melon.

In one specific form the fibre extracts from two fruits are used, the two fruit being orange and apple.

Citrus fruits that might be used including orange, grapefruit, tangelo, tangerine, lemon, kinnow fruit and varietals. When dealing with citrus by product parts, citrus "cups" can be used. Cups are halves of the outer portion of citrus fruits comprising the skin (flavedo) and the pith (albedo) and represent the portion of citrus fruit remaining after conventional juice extraction. The preferably starting material for fibre extraction is a shaved skin, whereby the flavedo has been removed. The benefit of using albedo is that processing is simplified, so that the strongly flavoured portion of the skin is not included.

For pineapples, the "zenith" solids, which comprise the outer skin and inner core of pineapple can be used. Also whole pineapples can be used.

When papaya is the precursor just the flesh and skin are to be processed. When the seed is included the resultant product has a higher fibre content. Likewise mangos, without seed can be processed.

When a melon is used it might be selected from the group consisting of watermelon, rock melon, honeydew melon or champagne melon.

The precursor material is preferably undigested, in the sense that it has not been macerated, or treated enzymically, or by other chemical agents such as acid or alkaline to breakdown the structure of the macromolecules forming the fibres. The structure of the plant material is thus still complex. Thus when an apple is prepared for pressing it is first milled, a process in which almost all of the cell walls are disrupted and in fact compounds normally isolated in cell walls or cytoplasm or vacuoles, nucleii etc are homogenised and begin to react . Many of these reactions are enzymically driven such as depectinisation or oxidation. On the other hand when an apple is prepared by a preferred embodiment of this invention the apple is sliced, so that the longest diffusion path is no more than say 1.5mm. Slicing disrupts only a small proportion of cell walls, perhaps 0.5%, and the enzymes and their substrates are kept separate.

The method of preparing fibre also preferably includes the step of inactivating enzymes within the fruit preparation which might conveniently be by heat inactivation. Thus with the example of apple slices after slicing the slices are flash heated to a temperature at which plasmolysis occurs but no heat damage occurs to flavour compounds (60°C).

This is termed a critical temperature. The resultant increase in permeability of the (still intact) cell wall increases significantly the rate of transfer of soluble solids from solid to liquid phase.

The majority of soluble solids are then removed from the precursor, by extracting liquids. This is achieved by comminuting the precursor material, to an appropriate size, for example with a particle with a thickness of no more than about 2 to 3 mm is found optimal for apple slices, and precontacting the precursor food material with an extraction liquid, and then separating the precursor food material from the extraction liquid, the separation occurring to an extent to give the desired reduction in soluble solids.

This extraction liquid is most preferably water, however, a non-aqueous or non-polar solvent might be used to extract water-insoluble or non-polar compounds. Examples of such solvents are, chloroform, hexane, chlorinated hydrocarbons or acetone. A specific example is the extraction of isoflavones and other flavanoids from orange peel using ethanol as the solvent.

It is preferred that water soluble solids are substantially all removed, in which case the fibre product is substantially free of sugars and other very readily soluble solids whereby greater than 90% of soluble solids are removed. One effect of this is that the fibre product is stabilised against microbial attack. That is not to say that microbial degradation of the fibre is totally inhibited, but rather that this is reduced. Generally fungal growth is not inhibited but growth of the more common food spoiling bacteria are.

Additionally by removal of substantially all of the soluble solids the fibre product has a reduced potential for the development of an off taste, because compounds responsible for flavours have been extracted by the extraction process. Removal of substantially all of the soluble solids is intended to mean removal of substantially all soluble solids that are in a free or unbound state.

A processor suitable for extraction by counter current methods is described in Australian Patent No. 543184. Alternatively other extraction apparatus that could be used include a diffuser made by Debanske Soccerfabriker of Denmark and a diffuser made by Amos of Germany. It is anticipated that by use of these processes greater than 90% of the water soluble solids are removed, and more preferably from between 93 to 99%.

The benefits of the invention are expected largely to result by reason of digestion in the large bowel of non-soluble components of the fibre component of the fruits outlined above, and it is anticipated that less purified forms of the fibres will also have a similar effect to that found for the more purified forms of fibre. It is however not desirable to use conventional techniques of expressing juice from fruit because the supplement will be high in flavours, sugars, and acids. The material is unstable microbiologically and enzymatically and will rapidly develop off flavours and odours and will quickly discolour.

It will be appreciated that the invention could also reside in a food product having the food supplement.

For a better understanding the invention will now be described with reference to a number of examples. It is understood that these examples are only illustrative and are not intended to limit the scope of the invention.

DETAILED DESCRIPTION OF EXAMPLES OF THE INVENTION.

PREPARATION OF APPLE FIBRE EXTRACT

Preparation

The apples were of the Granny Smith variety from Batlow in New South Wales, Australia, and were in good condition. Apples were prepared by slicing to a 2mm thickness with a crinkle cut to provide better structural integrity.

The Counter Current Extractor (CCE)

A counter current juice extractor available from CCT of Sydney Australia was used. The method of extracting juice from fruit and vegetables using this machine is described in Australia Patent No 543184. The CCE was set up with an angle of 4.5° a short cycle time of approximately 17 seconds, a residence time of about 1 hour. Oxidation was minimal at the temperature settings recorded.

The CCE was set up with the following operation conditions:-

- feed rate 12 kg/hr
- extraction water 15 kg/hr
- angle 4.5°
- cycle time 17 seconds
  - TF time forward 9.5 sec
  - TR time reverse 7.2 sec

• RPM	3
• Temperature at recycle	63°C
• Residence time	60 minutes
• Preparation	2mm slice (Crinkle cut)
• Pool level	low

Apples fed to the CCE via the slicer in 1 kg quantities at 5 minute intervals.

Extracted slices were recovered from the CCE in thoroughly cleaned plastic containers for further processing. Juice was recovered at a temperature of 18°C covered stainless steel buckets for further processing.

Fibre recovery

The fibre emerging from the CCE was collected and held for a period of about 4 hours then milled using a Fitzmill with 1/2 inch screen. This was to minimise damage to seed and skin tissue.

The fibre was then put through a paddle finisher to remove skin and seed tissue using a 40 thousandth of an inch screen where skin and seed tissue were removed. No attempt was made to dewater the fibre from this trial.

The resultant fibre was relatively free of seed and skin tissue but did contain some fine flakes of broken seed tissue. The yield of fibre emerging from the CCE represented 90% of the mass of the apples entering the process.

The loss of fibre at the cleaning stage of the process was in the order of 25% however these losses should be reduced to probably 5% at commercial scale.

Samples of the fibre were dried in an oven the results indicating that there was a recovery of 4.0 to 4.5% of the mass of the apples as dried fibre.

The quality of the fibre, organoleptically, was good being of pale colour and with no propensity to oxidize. It had only a very slight taste of apple which disappeared on drying, it was highly viscous (approximately 3 cm Bostwick) with strong water binding capacity.

Cleaned fibre was packed in heavy duty plastic bags in approximately 10kg quantities with a maximum thickness of 6cm. These packages were then stored at -20°C.

Yield of soluble solids in juice 92.9%. It should be noted that in commercial operation the extracted slices will be pressed to remove half their weight as water and this water (or dilute juice) is returned to the CCE as extraction liquor. Therefore yield equivalent is 96.5%. There was no evident browning of the fibre or juice emerging from the machine.

The resultant juice was very bright clear juice, pale in colour when compared to the spectrum of normal commercial products. This pale colour is viewed by the market as an advantage. The juice was free of pectin (alcohol test) and protein (heat test). The juice was of good aroma and flavour.

PREPARATION OF ORANGE FIBRE EXTRACT

Preparation

The peel used for this trial was from early season Valencia oranges grown in Berri in the Upper Murray district of South Australia.

Orange albedo was prepared in the following way at the Berri fruit juice plant at Berri, South Australia. Peel was returned from brown reamers to brown shavers where a gross separation of Albedo and Flavedo was effected. The separation was imperfect with the Albedo containing approximately 15% flavedo tissue. The two sections of peel from the shavers was packed into cardboard boxes each containing 10kg. Boxes of Flavedo and Albedo were then frozen to -20°C and transported to Sydney. Before feed to the CCE the Albedo tissue was thawed, further hand sorted to remove as much flavedo as possible and hand cut to reduce particle size (nominally 20mm x 3mm thick) using a knife.

CCE operation

The CCE was set up with the following operation conditions

- feed rate 12 kg/hr
- extraction water 15 kg/hr
- angle 7.0°
- cycle time 17 sec
  - TF time forward 9.5 sec
  - TR time reverse 7.2 sec
- RPM 3
- Temperature at recycle 75°C
- Residence time 60 minutes
- Preparation Hand slicing

• Pool level Low

Albedo tissue was fed to the CCE in 1 kg quantities at 5 minute intervals

The CCE was set up at a steep angle (7°) providing sufficient head to overcome the low porosity of the bed and the high viscosity of the extracting liquid. Relatively high temperatures were employed to minimise oxidative damage.

Fibre Handling

The extracted fibre emerging from the CCE was pressed with partial return of press liquor to increase the level of colour, then held for a period of about 2 hours at ambient temperature. Cleaned fibre was packed in heavy duty plastic bags in approximately 10 kg quantities with maximum thickness of 6cm. These packages were then stored at-18°C.

The fibre produced was of very pale yellow colour and mild bitterness but low in flavour and aroma. A small section of this was dried to constant weight in an over at 110°C. During this operation same maillard browning occurred although this was not severe and is not seen as a major barrier to commercialisation .

Juice

Yield of soluble solids in juice 75%. This yield was deliberately set as it is known that the partition coefficient for say limorin and naringim (bitter principals) between cellulose and orange juices is about 9. With higher yields of solubles unacceptable levels of bitter pincipals are extracted in the juice. However at a yield of 75% solubles, more than 50% of the bitter principle is carried out with the fibre.

The resultant juice was very bright but whiter in colour when compared to the spectrum of normal commercial orange products. The level of cloud was high. The juice had a viscosity of 18 cp at 12° brix and normal orange juice "mouthfeel". The juice was of good aroma and flavour with high sugar acid ratio (30:1)

PIG TRIALS OF FIBRE EXTRACTS

Materials and Methods

Animals

A total of 28 young-adult male pigs (starting live weight = 32 kg) were chosen for experiment and maintained in individual pens with a concrete floor in a temperature-

controlled room at the Pig Nutrition Research Facility (Roseworthy Campus). Pigs were obtained from the commercial piggery at the same institution.

Diets

Composition of the diets is shown in Table 1. The basal (Control) diet was formulated to be high in saturated fat (15% lipid by weight - 13% palm oil and 2% safflower oil), and marginal in calcium content (0.4%, by weight). Wheat bran was the source of dietary fibre (17% by weight, equivalent to 7.5% NSP). For treatment diets wheat bran was replaced by fibre extracts of Apple, Orange or Apple+Orange (in equal amounts)(see Table 1). Formulation of treatment diets was based on results of analyses for total dietary fibre of the two fibre extracts. Pigs were fed twice daily, at 0900 and 1600, at a rate proportional to their metabolic live weight (70 g x LW $^{0.75}$). The daily allowance was adjusted weekly when the animals were weighed. Pigs had unrestricted access to water for the duration of study.

Surgical Preparation and Experimental Design

For logistical reasons the study was split into two staggered sub-experiments (7-day overlap).

During the pre-experimental period, pigs were maintained on their regular commercial, pelleted diet for several days, until surgical implantation of a cephalic vein catheter. Pigs were then divided, on the basis of live weight, into 4 groups of six animals each. The remaining four animals were used as reserves. Immediately after surgery pigs were transferred to the Control diet (Day 7). After a further 7 days, three of the groups were randomly assigned to experimental diets, while the fourth group (and the unassigned animals) continued to be fed Control diet. Diets were fed for the remaining 21 days of experiment (days 7 to 28) and, at the end of the feeding period, pigs were anaesthetised in order to allow the designated samples to be collected, and then slaughtered.

Experimental Procedure and Measurements

Catheters were maintained by daily flushing of the dead-space with heparinised saline. Fasting blood samples were taken on 5 occasions (days 1, 7, 14, 21, and 28). The blood sample on Day 28 was taken from the abdominal aorta; all other blood samples were via cephalic vein catheter.

At the completion of the feeding period (day 28, - 21 days after the introduction of treatment diets) and approximately 16 hours after the pigs had been fed the evening before, they were weighed and anaesthetised (intravenous infusion of Pentothal). The abdominal cavity was opened and blood collected from the abdominal aorta, and the GI

tract was then ligated and excised, along with the liver. The liver was blot-dried and weighed, and a sample collected and snap-frozen. The small and large bowels were isolated and measured. The colon was divided into three segments of equal length, and the content of each of those segments, and that of the caecum, were extruded, weighed and sub sampled. The colon and caecum, devoid of contents, were blotted dry and weighed.

Small samples of liver and plasma were analysed for cholesterol content using gas chromatography. Digesta was diluted with distilled water for determination of pH and dry matter by standard techniques, and short-chain fatty acids (SCFA), caecal bile acids and neutral sterols by GC procedures.

Data Analysis

Data are shown as the mean and pooled standard error of the mean (SEM), with the number of observations per group as indicated in the tables. Statistical analysis was by one-way analysis - variance (ANOVA) and when significant values were detected (F value $P<0.05$), differences between individual means were then analysed by the PDIFF procedure of SAS. Differences between treatment means are considered significant at $P<0.05$. For tabulated results, values within a column with different superscripts differ significantly.

Hepatic and digesta metabolise pools were calculated as:

$$\text{Concentration } (\mu\text{mol/g or mmol/L}) \times \begin{cases} \text{weight of liver (g), or} \\ \text{volume of digesta water (mL).} \end{cases}$$

Results

Animal Health

During the pre-experimental period it was necessary to substitute a pig that had become lame for one from the reserve group. The catheters of two pigs in the Apple group ceased to function on about day 14 of the trial and therefore two additional pigs from the reserve group were assigned to this treatment. Consequently, at the time of slaughter, these two animals had been fed the treatment diet for just 14 days. However, as the results for these two animals were not substantially different from others in the group, they were therefore included in subsequent statistical analyses. One pig from the Apple+Orange group was euthanased just prior to completion of the study for reasons of illness (apparently unrelated to diet). During intubation (for catheterisation), several

pigs were found to have a throat infection, however this appeared to be a minor ailment and did not affect food consumption or rate of growth,

Food Acceptability and Live Weight Gain

Pigs found the diets acceptable, and there were no indications of overt adverse reactions. Pig growth rate (and feed conversion efficiency) during the period of study was satisfactory (average daily gain was ~500 g/d), and within the range encountered in commercial piggeries. Diet had no significant effect on live weight change during the feeding period (Table 2). The growth rate data is confirmation that there were no serious adverse reactions (eg diarrhoea, gastrointestinal disturbances or nutritional deficiencies) to these diets.

Small and Large Bowel Morphology

Neither intestinal length nor mass were influenced by dietary treatments (Table 3). Caecal and colonic weight were greatest in pigs fed Apple+Orange, however the differences were not statistically significant. Also, there were no differences between treatments for weight of the individual colonic segments (data not shown).

The Apple+Orange diet may have had a stimulatory effect on intestinal growth, as intestinal mass of the small bowel, caecum and colon were each greater numerically than that of any other treatment (but statistically not significant). Given that the colon of this group was about 10% heavier, and slightly shorter in length, than that of the others, it would suggest that the mixed fibre diet may have resulted in a thickening of the colonic wall. It is worth noting that the mixed fibre diet was particularly effective in raising SCFA levels at various sites in the large bowel (see later). These metabolises are potent trophic agents for intestinal mucosa.

Large Bowel Digesta Mass and Water Content

Generally, digesta mass was similar for the four groups (Table 4). Digesta mass of pigs fed wheat bran was greater at each of the large bowel sampling sites, however, these differences reached statistical significance for the mid colon only. Digesta moisture content declined progressively from the caecum through to the distal colon (Table 5). There were no significant treatment differences for this variable in the caecum and proximal colon, however, in the mid colon, water content of digesta in pigs fed Wheat bran was greater than that of the Apple treatment, but not significantly, compared to other treatments. Digesta in the distal colon of pigs given wheat bran contained about 10% more water than that for any of the other dietary treatments.

Similar amounts of wet digesta mass in the caecum of each group suggests that the amount of material entering the large bowel, which is primarily non-absorbed carbohydrate, is similar. For the Wheat bran group, there appears to be a progressive loss of material along the colon, however, for fibre extract treatments, fermentation occurs mainly in the proximal and mid colon. This finding reflects and confirms the highly fermentable nature of the fibre extract products. As large bowel bacteria catabolise fibre, its structure, and hence, water- holding capacity diminishes, along with the contribution that these materials could make to digesta mass and, hence, faecal weight. Although the fibre extracts would be expected to promote growth of enteric bacteria, this activity would occur primarily in the proximal region of the large bowel, and the contribution of expanded bacterial biomass to stool output may not be large. Data for the large bowel is compatible with the finding that the faeces of pigs fed diets containing fibre extracts, compared to Wheat bran, were much firmer, and formed into dense pellets. The apparent "constipating" effects of the fibre extract diets, however, do not appear to have been particularly serious (food intake, for example, was not compromised). Earlier studies have indicated that the optimal water content in digesta and faeces is 70- 80%. Also, because wheat bran is the most effective dietary fibre source for promoting stool weight (and alleviating constipation in human), differences with other fibres in relation to faecal output and consistency are expected.

Large Bowel pH

At each of the intestinal sampling sites, acidic conditions were found in large bowel lumen of those pigs receiving fibre extracts, compared to the Wheat bran. Differences in pH for the individual fibre extract treatments (Table 6) were not significant.

The fermentability of fibre extracts is clearly reflected in the acidic conditions found in the large bowel, especially in the caecum, and to a lesser extent, in the colon. Acidification of luminal contents has desirable health consequences, in that the formation, availability and absorption of various carcinogens and toxic materials in the hindgut is reduced. Indeed, a high pH in the human large bowel is thought to be a risk factor for colorectal cancer.

Large Bowel Short Chain Fatty Acid Concentrations

Generally, concentrations of total SCFA tended to be greatest in the caecum and proximal colon compared to the other sites, and throughout the large bowel, levels of these metabolises were higher in pigs fed fibre extracts (particularly for the diet containing mixed fibres) relative to Wheat bran, although only a few differences reached statistical significance (Table 7). Profiles for each of the individual SCFA were similar (Table 8-10). Compared to Wheat bran, the Apple+Orange treatment produced

substantially higher butyrate concentrations in the proximal and distal colon. Total and individual SCFA values for the mixed fibre treatment were (numerically) greater than those values obtained for either Apple or Orange fibre.

The finding that fibre extracts were effective in raising SCFA level is significant because of the role of these metabolises in the prevention and amelioration of important large bowel diseases. The mixed fibre diet was particularly effective in raising butyrate levels throughout the large bowel, especially in the distal colon, which is the site of most bowel disease in humans. The extent to which the level of SCFA and in particular butyrate has increased is quite surprising and is indicative of a synergy that has taken place between the fibre extracts. The exact nature of the synergy is unknown but it is expected that the synergy will also take place between fibre extracts of other fruit and vegetable sources. The effect of continued consumption of the mixed fibre product however is suggested to enhance the microflora in the large intestine that are capable of producing SCFA and thereby reducing the population of microflora that lead to adverse health effects.

The fibre extracts used are convenient to handle because they are fermentable in the large bowel, and are not heat labile.

It is thought that the high pectin content of the two fibre extracts used provides a fermentable fibre but the nature of the synergy is not clear at present.

CORNFLAKE FORMULATION

An example of a food product to which the apple and orange fibre mix, in the proportions et out above might be added is set out below.

A corn flake formulation is as follows:

	Normal	With fibre mixture
Maize flour	91%	77%
Fibre mixture	-	14%
Malt	3%	3%
Sugar	5%	5%
Salt	1%	1%

The process of making the corn flake product with fibre mixture is the same as making the cornflake mixture with the normal mix and is in accordance with methods known to the person skilled in the art.

Various features of the invention have been particularly shown and described in connection with the exemplified embodiment of the invention, however, it must be understood that these particular arrangements merely illustrate and that the invention is not limited thereto and can include various modifications falling within the spirit and scope of the invention.

Dated this 14th day of April 1998

ALLRAD 3 PTY LTD AND
PTI INVESTMENTS PTY LTD
By their Patent Attorneys,
A. P. T. Patent and Trade Mark Attorneys

TABLE 1
Composition of experimental diets (g/kg)

Ingredient	Control	Apple	Orange	Apple+Orange
Casein	160	143	144	143
Starch	497	497	497	497
Sugar	100	53	58	55
Palm oil	130	188	188	188
Safflower oil	20	20	20	20
Wheat bran	75	-	-	-
Apple extract	-	106	-	53
Orange extract	-	13.3	96	48
Dicalcium phosphate	13.3	13.3	13.3	13.3
Minerals (other)	3.2	3.2	3.2	3.2
Vitamins	1.5	1.5	1.5	1.5

TABLE 2
Initial and final live weight, and live weight gain of pigs

Dietary group	Live Weight		Live Weight Gain
	Initial	Final	
		kg	
Wheat bran	31.6	48.1	16.5
Apple	31.8	46.1	14.2
Orange	33.2	47.8	14.6
Apple+Orange	33.5	48.7	15.2
SEM	1.3	1.8	1.0

Values are means for 7, 8, 6 and 5 observations for treatments Wheat bran, Apple, Orange and Apple+Orange, respectively.
Treatment differences are not statistically significant ($P<0.05$).

TABLE 3
Morphology of the small and large bowel

Dietary group	Small Intestine	Caecum		Colon	
	Length	Weight	Length	Weight	Length
	m	g	cm	g	m
Wheat bran	15.29	95.9	16.7	568	2.97
Apple	15.15	101.5	15.6	550	2.84
Orange	14.65	89.7	14.8	547	2.81
Apple+Orange	15.32	109.8	15.2	613	2.77
SEM	0.51	9.4	1.0	34	0.12

Values are means, with number of observations as per Table 5.

TABLE 4
Wet weight of digesta in the large bowel

Dietary Group	Caecal	Proximal colon	Mid colon	Distal colon	Total colon
			g		
Wheat bran	152	349	216[a]	154	871
Apple	135	252	117[c]	107	611
Orange	169	274	118[b]	81	642
Apple+Orange	143	243	129[b]	112	627
SEM	37	39	27	22	90

Values are means, with number of observations as per Table 2. Means in the same column with different superscript letters differ ($P<0.05$). [a-b], $P<0.05$, [a-c], $P<0.01$.

TABLE 5
Water content of digesta in the large bowel

Dietary Group	Caecal	Proximal colon	Mid colon	Distal colon
			%	
Wheat bran	88	84.4	79.8[a]	73.2[a]
Apple	90.4	83.6	74.5[b]	63.2[c]
Orange	88.4	82.1	76.5	63.9[c]
Apple+Orange	91.0	84.0	76.0	62.8[c]
SEM	2.1	2.0	2.0	2.0

Values are means, with number of observations as per Table 5. Means in the same column with different superscript letters differ [a-b], $P<0.05$; [a-c], $P<0.01$.

TABLE 6
pH of digesta in the large bowel

Dietary Group	Caecal	Proximal colon	Mid colon	Distal colon
Wheat bran	7.13[a]	7.10[a]	7.21[a]	7.09[a]
Apple	5.84[d]	6.18[d]	6.24[d]	6.29[d]
Orange	5.84[d]	6.09[d]	6.14[d]	6.28[d]
Apple+Orange	5.85[d]	6.06[d]	6.06[d]	6.05[d]
SEM	0.16	0.11	0.13	0.13

Values are means, with number of observations as per Table 2. Means in the same column with different superscript letters differ [a-b], $P<0.05$; [a-c], $P<0.01$, [a-d], $P<0.01$.

TABLE 7
Concentration of total SCFA in the large bowel

Dietary Group	Caecal	Proximal colon	Mid colon	Distal colon
		mmol/L		
Wheat bran	62.8	60.5[a]	53.1	55.4
Apple	68.3	71.1[a]	55.4	43.7
Orange	75.5	79.9[ac]	67.6	42.5
Apple+Orange	88.2	93.3[c]	76.3	61.7
SEM	15.7	7.3	8.3	6.7

Values are means, with number of observations as per Table 2. Means in the same column with different superscript letters differ: [a-c], $P<0.01$.
For the Proximal colon, Wheat bran vs Orange, $P=0.07$; for Mid colon and Distal colon, ANOVA F value not significant.
For Mid colon, Wheat bran vs Apple+Orange, $P=0.073$; Apple vs Apple+Orange, $P=0.095$. For Distal colon, Apple+Orange vs Apple, $P=0.074$, Apple+Orange vs Orange, $P=0.074$.

TABLE 8
Concentration of total acetate in the large bowel

Dietary Group	Caecal	Proximal colon	Mid colon	Distal colon
		mmol/L		
Wheat bran	40.8	38.6a	33.7	35.2
Apple	43.0	42.4[bc]	33.3	27.0
Orange	48.6	48.5	39.6	26.6
Apple+Orange	59.2	60.1[c]	46.2	36.7
SEM	10.2	4.8	5.3	4.2

Values are means, with number of observations as per Table 2.
Means in the same column with different superscript letters differ: [a-b], $P<0.05$; [a-c], $P<0.01$.

TABLE 9
Concentration of propionate in the large bowel

Dietary Group	Caecal	Proximal colon	Mid colon	Distal colon
		mmol/L		
Wheat bran	17.3	15.7	12.0	12.6
Apple	19.5	19.6	12.9	8.38
Orange	21.2	22.6	18.1	8.73
Apple+Orange	21.2	22.7	19.1	11.9
SEM	4.4	2.3	2.7	1.7

Values are means, with number of observations as per Table 2.
ANOVA F value not significant.
For Mid colon, Wheat bran vs Apple+Orange, P=0.085.
For Distal colon, Wheat bran vs Apple, P=0.076.

TABLE 10
Concentration of butyrate in the large bowel

Dietary Group	Caecal	Proximal colon	Mid colon	Distal colon
		mmol/L		
Wheat bran	4.74	6.06	7.43	7.67[a]
Apple	5.83	9.15	9.20	8.28[a]
Orange	5.73	8.80	9.90	7.13[a]
Apple+Orange	7.80	10.4	11.0	13.0[b]
SEM	1.62	1.1	1.4	1.4

Values are means, with number of observations as per Table 2. Means in the same column with different superscript letters differ: ANOVA F value not significant for Caecum, Proximal and Mid colon. For Proximal colon, Wheat bran vs Apple, P=0.052.